REMARKS

This responds to the Office Action mailed on September 13, 2006.

Claims 18, 47, 83, 90, and 95-96 are amended. Claims 1, 3-6, 9, 11-12, 15, 18, 20-21, 24-39, 41-45, 47, 60, 64, 67, 69-71, 74, 76-78, 80-88, and 90-96 are pending.

Claims 90, 95 and 96 were objected to because of the following informality: the omission of the word "to" after "selected". The amendments to claims 90 and 95-96 obviate this objection.

The Nonstatutory Obviousness-Type Double Patenting Rejection

Claims 91, 93 and 94 were provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-50 and 58-60 of copending application Serial No. 10/314,827. Applicant notes that neither the present application nor the '827 application has issued. Therefore, a terminal disclaimer is not required in these matters until issuance of one of them. If a terminal disclaimer is required for either application, it can be requested by the Examiner before issuance.

The 35 U.S.C. § 112, Second Paragraph, Rejections

Claim 18 was rejected under 35 U.S.C. § 112, second paragraph, as being confusing in the recitation "or the complement thereof which encodes a luciferase". The amendment to claim 18 renders this rejection moot.

Claims 47 and 83 were rejected under 35 U.S.C. § 112, second paragraph, as indefinite in the recitation of "corresponding wild type nucleic acid sequence" as it is unclear to what sequence this refers. Claim 83 was also rejected under 35 U.S.C. § 112, second paragraph, as confusing for the recitation of SEQ ID NO:22. The amendments to claims 47 and 83 obviate these § 112(2) rejections.

Claims 1, 3-6, 9, 11-12, 15, 18, 20-21, 24-39, 41-45, 47, 60, 67, 69-71, 74, 76-78, 80-83, 85-88, and 90-96 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. This rejection is respectfully traversed.

It is Applicant's position that the phrases "transcription factor binding sequences," "intron splice sites," "poly(A) addition sites" and "prokaryotic 5' noncoding regulatory sequences" such as promoter sequences are conventionally used and understood by the art. See, e.g., U.S. Patent No. 5,670,356 ("transcription factor binding sites"), WO 97/47358 ("intron splice sites"), Iannaconne et al., Plant Mol. Biol., 34:485 (1997) ("polyA sequences"), and Pan et al., Nucl. Acids Res., 27:1094 (1999) ("prokaryotic promoters," "poly(A) signals," and "exon-intron boundaries") (all of record), and Faisst and Meyer, Nucl. Acids Res., 20:3 (1992) (cited at page 50, lines 6-7 of Applicant's specification), which discloses a compilation of vertebrate encoded transcription factors. Moreover, the Examiner acknowledges that those terms are conventional in the art (page 3 of the Office Action).

Further, each of the recited classes of sequences or sites has a definite property that is recognizable (and testable) by one of skill in the art. For instance, a poly(A) addition site is a sequence where polyadenylation occurs at the end of a transcript; a promoter is a sequence that binds proteins and directs transcription of a gene; a splice site is a sequence that signals where a primary RNA transcript is to be spliced to form a mRNA; and a transcription factor binding sequence is a sequence that binds a transcription factor, a protein that alters transcription.

The Examiner also asserts that the phrases "wherein the mammalian transcription factor binding sequences are those present in a database of transcription factor binding sequences" and "known mammalian transcription factor binding sequences" are vague and indefinite. It is Applicant's position that one of skill in the art is aware of databases having transcription factor binding sequences, e.g., see Applicant's specification and the Wood Declaration mentioned below, or aware of other sources of mammalian transcription factor binding sites (e.g., Faisst and Meyer, Nucl. Acids Res., 20:3 (1992), cited at page 50, lines 6-7 of Applicant's specification).

Therefore, one of skill in the art would understand the metes and bounds of "mammalian transcription factor binding sequences," "intron splice sites," "poly(A) addition sites", "prokaryotic 5' noncoding regulatory sequences" , "wherein the mammalian transcription factor binding sequences are those present in a database of transcription factor binding sequences" and "known mammalian transcription factor binding sequences".

With regard to calculating the number of mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory

sequences, since those sequences can be identified, the number present in a polynucleotide can likewise be calculated. The Examiner is requested to consider that Example 1 in the above-identified specification discloses that synthetic click beetle luciferase sequences were prepared that had a reduced number of a combination of mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences.

For instance, it is disclosed that mammalian codon replacement in a parent click beetle luciferase sequence yielded a mammalian codon optimized click beetle luciferase sequence (GRver1). Removal of intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences, e.g., promoter sequences, in the mammalian codon optimized click beetle luciferase sequence by codon replacement, resulted in a sequence, GRver2, that had about 100 mammalian transcription factor binding sequences. Replacement of codons in GRver2 to remove those mammalian transcription factor binding sequences, and intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences, yielded a sequence, GRver3, that had about 50 newly introduced mammalian transcription factor binding sequences. Replacement of codons in GRver3 to remove those mammalian transcription factor binding sequences, and intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences, yielded a sequence, GRver4, that had about 20 newly introduced mammalian transcription factor binding sequences. Those newly introduced mammalian transcription factor binding sequences were removed by codon replacement to yield GRver5. It is disclosed that GRver5 was devoid of eukaryotic transcription factor binding sequences but did contain one splice acceptor sequence.

Moreover, as described in the Rule 132 Declaration filed on June 19, 2006 and executed by Monika Wood, a co-inventor of the above-referenced application, using software and a database that are available to the public and comparable to those disclosed in the application, she determined the number of mammalian transcription factor binding sequences in *luc*+, a sequence described in Sherf et al. (U.S. Patent No. 5,670,356), a reference cited against the claims under 35 U.S.C. § 103(a).

Thus, the calculation of the number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic 5' noncoding regulatory sequences

(prokaryotic promoter sequences hereinafter) in a particular sequence is possible and can be determined by one of skill in the art.

With regard to the alleged change in scope of claims which recite mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and promoter sequences, it is Applicant's position that intron splice sites, poly(A) addition sites, and prokaryotic promoter sequences represent relatively conserved sequences that were well known prior to Applicant's effective filing date (Mount, *supra*; Jensen et al., *supra*; Hsieh et al., *supra*; and Andrews et al., *supra*). And although there may be new members added to the group "mammalian transcription factors" over time, the independent claims in the present application provide that the synthetic nucleic acid molecules (polynucleotides) have a reduced number of a combination of mammalian transcription factor binding sequences, as a result of codon replacement of at least 25% of the codons of a parent reporter nucleic acid sequence with mammalian high usage codons and mammalian codons that are not high usage.

Thus, Applicant's synthetic polynucleotides are readily recognized by one of skill in the art. That is because they are reporter encoding polynucleotides with a large number of mammalian codons relative to a corresponding wild-type (or parent) polynucleotide, and a reduction in a combination of mammalian transcription factor binding sequences, as well as optionally intron splice sites, poly(A) addition sites and prokaryotic promoter sequences. The presence of the large number of mammalian codons and the reduction in a combination of mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and/or prokaryotic promoter sequences, result in the synthetic polynucleotides being significantly divergent in nucleotide sequence relative to the corresponding wild-type or parent polynucleotide. Moreover, the synthetic polynucleotides include mammalian codons which are not "high usage" mammalian codons, as those codons replace codons that result in mammalian transcription factor binding sequences, intron splice sites, poly(A) addition sites and/or prokaryotic promoter sequences.

Accordingly, withdrawal of the 35 U.S.C. § 112, second paragraph, rejections is respectfully requested.

The 35 U.S.C. § 112, First Paragraph, Rejection

Claims 1, 3-6, 9, 11-12, 15, 20-21, 24-33, 35-39, 41-45, 47, 60, 67, 69-70, 81-82, 86-88, and 90-95 were rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement. This rejection is respectfully traversed.

The Examiner asserts that if the skilled artisan cannot identify transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic promoter sequences, the skilled artisan cannot practice the invention.

The Examiner is requested to consider that it was well within the skill of the art to identify transcription factor binding sequences, intron splice sites, poly(A) addition sites and prokaryotic promoter sequences. See Mount, *supra*; Jensen et al., *supra*; Hsieh et al., *supra*; and Andrews et al., *supra*. Moreover, it is within the skill of the art to test whether a particular sequence binds transcription factors, is a splice donor or splice acceptor, is a poly(A) addition site, or initiates transcription in a prokaryotic system.

The Examiner also asserts that the specification does not reasonably provide enablement for any variant DNA molecules encoding any reporter polypeptide having at least 90% identity to a wild type reporter polypeptide, having more than 25% of the codons altered and having a reduced number of transcription factor binding sequences, intron splice sites, poly(A) addition sites and 5' noncoding regulatory sequences than a mammalian codon optimized version of the parent nucleic acid or to any nucleic acid which will hybridize to SEQ ID NO:9 under medium stringency conditions.

With respect to variants of reporter polypeptides, such as GFP, beetle luciferase, GUS, and CAT, as well as beta-lactamase, Applicant has provided evidence that it is well within the skill of the art to introduce substitutions into various reporter proteins and yield a variant protein with the activity of the corresponding wild-type reporter protein (see, e.g., the abstracts for Stapleton et al., Antimicrob. Agents Chemother., 43:1881 (1999); Bouthors et al., Protein Eng., 12:313 (1999); Sirot et al., Antimicrob. Agents Chemother., 41:1322 (1997); Voladri et al., J. Bacteriol., 178:7248 (1996); Murray et al., J. Mol. Biol., 254:993 (1995); and Matsumura et al., Nat. Biotechnol., 17:696 (1999), and U.S. Patent No. 5,874,304; all of record).

In particular, with regard to luciferases, numerous substitutions have been introduced into beetle luciferases without affecting the reporter property of the substitution variants (see, e.g.,

Kajiyama et al., Protein Engineering, 4:691 (1991)), Wood et al., J. Biolumin., 4:31 (1989), Wood et al., J. Biolumin., 5:107 (1990) and Sala-Newby et al., Biochem. J., 279:727 (1991)), U.S. Patent Nos. 5,670,356, 6,552,179, 6,387,675 and 6,602,677 (all of record). Note that LucPpyYG (SEQ ID NO:23) and YG#81-6G01 (SEQ ID NO:24) have over 95% amino acid sequence identity to each other and both function as reporters. Further, in U.S. Patent No. 6,602,677, five mutant luciferases are disclosed that have 12, 21, 32, 37 and 37 substitutions, respectively, relative to a parent luciferase, and functions as reporters. Also, the Examiner is requested to note that in Example 1 of the above-referenced application, the amino acid sequence of the click beetle luciferases encoded by synthetic nucleic acid sequences of the invention is different than the amino acid sequence of the parent click beetle luciferase. Similarly, in Example 3 of the present application, the amino acid sequence of the *Renilla* luciferase encoded by a synthetic nucleic acid sequence is different than the amino acid sequence of the parent *Renilla* luciferase sequence.

The Examiner also asserts that firefly luciferases and click beetle luciferases differ from each other. Nevertheless, the primary amino acid sequences of firefly luciferases and click beetle luciferases have common features, and so can be aligned (see Figure 8 in Wood et al., J. Biolum. Chemi., 4:289 (1989), and Figure 3 in Wood et al., Science, 244:700 (1989); of record). Such an alignment, in view of positions that have been substituted in beetle luciferases, provides direction on what residues may be substituted without altering beetle luciferase reporter properties.

Accordingly, the substitution of residues in reporter proteins such as luciferases that result in a variant with reporter activity is well within the skill of the art.

With regard to the Examiner's assertion that it is not routine to screen for multiple substitutions or multiple modifications, the Examiner is requested to consider WO 99/14336, a reference previously cited against the claims under 35 U.S.C. § 103(a), and Arnold (Chem. Eng. Sci., 51:5091 (1996) (of record) which discloses the introduction of multiple modifications into a nucleic acid molecule and screening for particular phenotype(s) of the encoded gene product.

In response to the undue experimentation alleged to be necessary to prepare variant reporters and screen those reporters for activity, the fact that the outcome of such a screening program may be unpredictable is precisely why a program is carried out. The Examiner simply

cannot reasonably contend that a program to locate biomolecules with target biological or physical properties would not be carried out by the art because the results cannot be predicted in advance.

In fact, the Federal Circuit has explicitly recognized that the need, and methodologies required, to carry out extensive synthesis and screening programs to locate biomolecules with particular properties do not constitute undue experimentation. In re Wands, 8 U.S.P.Q.2d 1400, 1406-1407 (Fed. Cir. 1988), the Court stated:

> The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody.

Likewise, practitioners in the art related to the present application would be well-equipped to prepare and/or screen variant reporter constructs to identify those with reporter activity. See also, Hybritech Inc. v. Monoclonal Antibodies Inc., 231 U.S.P.Q. 81, 84 (Fed. Cir. 1986) (evidence that screening methods used to identify characteristics [of monoclonal antibodies] were available to art convincing of enablement). Thus, the fact that a given claim may encompass a variety of molecules is not dispositive of the enablement issue, particularly in an art area in which the level of skill is very high and in which screening of large numbers of compounds has been standard practice for at least ten years (Ex parte Forman, 230 U.S.P.Q.2d 456 (Bd. App. 1986).

In this regard, the Examiner is also requested to consider that prior to Applicant's filing, the screening of mutant nucleic acid libraries for proteins with selected properties was routine (see the abstracts for Kim et al., Appl. Envir. Micro., 66:788 (2000); Xu-Welliver et al., Cancer Res., 58:1936 (1998); Zhang et al., Metab. Eng., 2:339 (2000), (a copy of each is enclosed herewith).

The Examiner asserts that while methods of determining if any individual sequence would have the properties described are well known in the art, methods of determining which sequences from the virtually infinite genus of sequences capable of hybridizing to under medium stringency conditions to the recited nucleic acids and encoding a protein having 90% identity to

any beetle luciferase actually are within the scope of the instant claims (i.e., encode a luciferase protein) beyond just making and testing all possibilities, are not provided.

First, it is Applicant's position that one of skill in the art in possession of Applicant's specification is readily able to determine whether a variant nucleic acid molecule hybridizes under medium stringency conditions to Applicant's synthetic polynucleotides, e.g., hybridizes to SEQ ID NO:9. Moreover, if one of skill in the art in possession of Applicant's specification is readily able to determine whether a variant nucleic acid molecule hybridizes under high stringency conditions to Applicant's synthetic polynucleotides (notes claims 71 and 83-85 are not rejected under § 112(1)), it is logical to conclude that the art worker can determine whether the variant nucleic acid molecule also hybridizes under medium stringency conditions.

Thus, Applicant's specification is enabling.

Therefore, withdrawal of the § 112(1) rejection is appropriate and is respectfully requested.

The 35 U.S.C. § 103 Rejections

Claims 1, 3-6, 9, 11-12, 15, 20-21, 24-39, 41-45, 60, 67, 69-70, 81, 86, and 90-95 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Sherf et al. (U.S. Patent No. 5,670,356) in view of Zolotukhin et al. (U.S. Patent No. 5,874,304), Donnelly et al. (WO 97/47358), Pan et al. (Nucl. Acids Res., 27:1094 (1999)), Cornelissen et al. (U.S. Patent No. 5,952,547), and Hey et al. (U.S. Patent No. 6,169,232). Claims 18, 47, 71, 74, 76-78, 80, 82-85, 87-88, and 96 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Sherf et al. in view of Zolotukhin et al., Donnelly et al., Pan et al., Cornelissen et al., and Hey et al., and further in view of Wood et al. (WO 99/14336). These rejections are respectfully traversed.

Sherf et al. disclose a synthetic firefly luciferase gene (*luc*$^{+}$) in which 3 internal palindromic sequences, 5 restriction endonuclease sites, 4 glycosylation sites, and 6 transcription factor binding sites that were present in the unmodified sequence were removed. Also codons were altered at sequences specified in Table 2 to codons preferred ("more common") in mammalian cells, relative to a wild type firefly luciferase gene (*luc*). Of the twenty 6 to 30 bp regions which were modified, 6 regions included modifications with a dual purpose, i.e., one region was modified to eliminate a glycosylation site and a transcription factor binding site that

was present in the unmodified sequence, three regions were modified to eliminate a transcription factor binding site that was present in the unmodified sequence and improve codon usage, one region was modified to eliminate two transcription factor binding sites (but not improve codon usage) that were present in the unmodified sequence, and another region was modified to improve codon usage and eliminate a restriction endonuclease recognition site.

Sherf et al. also disclose that a vector encoding Luc^+ or Luc was introduced to four mammalian cell lines. NIH3T3 and HeLa cells transfected with *luc*$^+$ DNA had significantly higher levels of luciferase activity relative to NIH3T3 and HeLa cells transfected with *luc* DNA (Table 3), while CHO and CV-1 cells transfected with *luc*$^+$ or *luc* DNA had comparable luciferase activity. However, it is unclear what alterations in *luc*$^+$ DNA increased luciferase activity in mammalian cells, and why those alterations did not uniformly increase luciferase activity in all the tested mammalian cells.

In contrast, a synthetic *Renilla* luciferase gene of the present invention was expressed at significantly higher levels relative to a wild type *Renilla* luciferase gene in NIH3T3, HeLa, CHO and CV-1 cells (Table 10).

Sherf et al. do not teach or suggest that modification of a parent sequence to remove palindromic sequences, restriction endonuclease sites, glycosylation sites, and transcription factor binding sites may introduce other undesirable sequences. Nor do Sherf et al. disclose or suggest replacing at least 25% of the codons in a parent sequence with selected mammalian codons, thereby reducing a large number of transcription factor binding sequences in the parent sequence.

A humanized version of a green fluorescent protein (GFP) gene is disclosed in Zolotukhin et al. in which 88/238 of the codons in the gene were altered (column 13, lines 1-4). Zolotukhin et al. do not disclose or suggest that codon optimization of a parent sequence may introduce undesirable sequences.

WO 97/47358 discloses the preparation of synthetic hepatitis C virus (HCV) genes. In particular, it is disclosed that codons in the corresponding wild-type gene that are not the most commonly employed in humans, are replaced with an optimal codon. Of note, HCV is a pathogen of humans (only humans and chimpanzees are susceptible to infection, see

www.who.int/mediacentre/factsheets; copy enclosed) and so due to evolutionary selection, HCV sequences are likely at least partially human codon optimized.

WO 97/47358 also discloses that if a CG is created by that codon replacement, i.e., the third nucleotide in the replaced codon is C and the first nucleotide in the adjacent codon is G, WO 97/47358 discloses that a different codon is selected based on Table 5 in Lathe et al. (J. Mol. Biol., 183:1 (1985)) (page 17). Once all codon replacements are made, it is disclosed that the codon optimized gene is inspected for undesired sequences such as ATTTA sequences, inadvertent creation of intron splice sites, and unwanted restriction enzyme sites, which are then eliminated by substituting codons (pages 17-18).

The bias away from CG residues during codon optimization in WO 97/47358 would reduce overall CG content in the final synthetic sequence unless codon substitution to remove undesired sequences resulted in an increase in CG dinucleotides in adjacent codons (thus defeating the reasoning behind avoiding CGs in adjacent codons). In that regard, note that the synthetic click beetle and *Renilla* luciferase genes described in the Examples had increased CG content relative to the respective parent sequence.

WO 97/47358 provides no details of the sequence of any undesirable sites including intron splice sites which are to be eliminated or how to substitute codons to remove ATTTA sequences, splicing sites and restriction enzyme sites. Further, there is no recognition in WO 97/47358 that codon optimization may introduce transcription factor binding sequences or that transcription factor binding sequences may be removed from sequences.

Pan et al. describe a synthetic gene derived from the merozoite surface protein-1 gene (*msp*-1) of *Plasmodium falciparum*. Note that the life cycle of *P. falciparum* includes humans and mosquitos, and that this pathogen replicates in human hepatocytes and erythrocytes. Thus, due to evolutionary selection, *P. falciparum* sequences are at least partially human codon optimized.

The synthetic gene in Pan et al. was prepared by first back translating the corresponding wild type gene using random (not preferred) human codon replacement. One master sequence was chosen, and then the master synthetic sequence was modified via alternate codon replacement to eliminate sequences that might be detrimental to efficient transcription and translation, i.e., endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, exon-intron

boundaries, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, and long runs of purines (page 1095).

Notably, Pan et al. did not seek to eliminate transcription factor binding sequences in *msp*-1 and did not recognize that codon optimization may introduce transcription factor binding sequences. Nor does Pan et al. disclose the sequences for prokaryotic promoters, poly(A) signals, or exon-intron boundaries that are required to be identified for removal.

Cornelissen et al. relate that DNA encoding a Bt crystal protein (insect protein) is modified by changing A and T sequences to G and C sequences encoding the same amino acids (Abstract), in a region that would otherwise have a low percentage of RNA polymerase II compared to an adjacent, upstream region (page 6), to improve expression in plant cells. It is disclosed that *in vitro* binding assays with proteins from tobacco nuclei showed binding in a region 700 to 1000 nucleotides downstream of the transcriptional start site (page 10). It is disclosed that, in particular, a 29 bp region in a 268 bp fragment between positions 733 and 1000 in the Bt protein coding sequence has two sequences which may reduce elongation efficiency. A 326 bp fragment in the Bt protein coding region was replaced with one having 59 codons modified by changing A and T sequences to G and C sequences (page 12).

Cornelissen et al. do not disclose or suggest modifying reporter sequences, e.g., luciferase sequences, or recognize that codon replacement may introduce undesirable sites. Nor do Cornelissen et al. disclose or suggest replacing codons in a gene with mammalian codons.

Hey et al. disclose altering codons in storage proteins to yield sink protein nucleic acid sequences that have Trp codons for Phe codons (to increase the nutritional value of seed) and also have a reduction in splice sites, polyA sequences, RNA polymerase termination signals, TA and CG doublets and blocks of G or C residues of more than about 4 residues (column 2, lines 53-67 and column 6, lines 22-25). It is disclosed that a sink protein sequence was back translated, codons preferred in maize introduced (column 10, lines 55-66), and restriction enzyme sites, splice sites, polyA sequences, RNA polymerase termination signals, TA and CG doublets and blocks of G or C residues of more than about 4 residues replaced with second or third choice codons (column 11, lines 2-15).

Hey et al. do not disclose or suggest modifying reporter sequences, or recognize that mammalian codon replacement introduces mammalian transcription factor binding sites that, in turn need to be removed.

WO 99/14336 discloses thermostable beetle luciferases and a method to prepare those luciferases. It is disclosed that the thermostable beetle luciferases have a plurality of amino acid substitutions relative to a wild-type beetle luciferase, and that they may be prepared by iterative mutagenesis and selection methods.

In order for the Examiner to establish a *prima facie* case of obviousness, three base criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on Applicant's disclosure. M.P.E.P. § 2142 (citing In re Vaeck, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

The combination of references does not disclose or suggest Applicant's invention as each reference discloses a different way to modify the coding sequence of a different gene to increase expression, i.e., viral genes, a gene from a parasite associated with malaria, an insect toxin gene, a storage protein gene, or a reporter gene. That is, Zolotukhin et al. disclose codon modification alone generally throughout a green fluorescent protein gene to codons employed more frequently in one organism, and Sherf et al. disclose limited and targeted modification (modifications in 20 regions of 6 to 30 bp) of a firefly luciferase sequence to introduce or remove cloning sites, alter insect codons to mammalian codons, and to remove post-translation modification sites, secondary structure, and transcription factor binding sites. Cornelissen et al. disclose targeted modification of a toxin gene to alter *Bacillus* codons to remove sequences that may alter elongation efficiency, e.g., by replacing A and T sequences with G and C sequences, WO 97/47358 describes codon replacement to more commonly employed codons combined with further codon substitution to remove CG residue in adjacent codons, and then inspects for ATTTA sequences, intron splice sites, and unwanted restriction enzyme sites. Hey et al.

describe codon replacement in storage protein genes to maize codons and reducing restriction enzyme sites, splice sites, polyA sequences, RNA polymerase termination signals, TA and CG doublets, and blocks of G or C residues. Pan et al. disclose random human codon replacement yielding a population of synthetic sequences with codon substitutions, choosing one master synthetic sequence, and then modifying the master synthetic sequence via alternate codon replacement to eliminate sequences that might be detrimental to efficient transcription and translation, i.e., endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, exon-intron boundaries, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, and long runs of purines, and WO 99/14336 discloses modified thermostable beetle luciferases prepared by mutagenesis leading to a plurality of amino acid substitutions, followed by selection for one or more phenotypes.

Thus, while there is a general teaching in the combination of cited documents to alter codons and/or remove certain undesired sequences in a selected sequence, none of the cited documents teaches or suggests that codon alterations, optionally in conjunction with removal of ATTTA sequences, splice sites, endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, long runs of purines, RNA polymerase termination signals, TA and CG doublets and blocks of G or C residues of more than about 4 residues, may create transcription factor binding sites. Moreover, none of the cited documents discloses or suggests reiterative removal of transcription factor binding sites from a codon optimized gene of any type.

And although one of skill in the art in possession of the cited documents may be motivated to alter the codons of a particular sequence, there is no direction in the combination of cited documents which directs one of skill in the art to Applicant's invention. It is only with hindsight, i.e., with knowledge of Applicant's invention, that one of skill in the art, picking and choosing from the cited documents, may be directed to Applicant's invention. That is, with regard to Applicant's synthetic reporter encoding polynucleotides (claims 1, 3-6, 9, 11-12, 15, 20-21, 24-39, 41-45, 60, 67, 69-70, 81, 86, and 90-95), one of skill in the art in possession of the cited art, would be required to modify a reporter gene (Sherf et al. and Zolotukhin et al.), rather than a non-reporter gene (WO 97/47358, Cornelissen et al., Hey et al., and Pan et al.), by codon replacement over an entire opening reading frame (Zolotukhin et al., WO 97/47358, Hey et al.

and Pan et al.) rather than by alterations in a portion of an open reading frame (Sherf et al. and Cornelissen et al.), with subsequent additional directed alterations to the nucleotide sequence to remove undesired sequences introduced by codon replacement (WO 97/47358, Hey et al. and Pan et al.) rather than a lack of substantive subsequent additional directed alterations to the nucleotide sequence to remove undesired sequences introduced by codon replacement or concurrently with other alterations, or via selection (Sherf et al., Zolotukhin et al., and Cornelissen et al.), where codons are first replaced with preferred mammalian codons (Sherf et al., Zolotukhin et al., and WO 97/47358), rather than random codons (Pan et al.), plant codons (Hey et al.), or G and C residues (Cornelissen et al.), and in some instances, codons are further replaced to remove ATTTA sequences, splice sites, endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, long runs of purines, RNA polymerase termination signals, TA and CG doublets and blocks of G or C residues of more than about 4 residues (WO 97/47358, Pan et al. and Hey et al.)

With regard to Applicant's synthetic luciferase encoding polynucleotides (claims 18, 47, 71, 74, 76-78, 80, 82-85, 87-88, and 96), one of skill in the art in possession of the cited art, would be required to modify a luciferase gene (Sherf et al. and WO 99/14336), rather than a non-luciferase gene (Zolotukhin et al., WO 97/47358, Cornelissen et al., Hey et al., and Pan et al.), by codon replacement over an entire opening reading frame (Zolotukhin et al., WO 97/47358, Hey et al. and Pan et al.) rather than by alterations in a portion of an open reading frame (Sherf et al. and Cornelissen et al.) or by mutagenesis and selection (WO 99/14336), with subsequent additional directed alterations to the nucleotide sequence to remove undesired sequences introduced by codon replacement (WO 97/47358, Hey et al. and Pan et al.) rather than a lack of substantive subsequent additional directed alterations to the nucleotide sequence to remove undesired sequences introduced by codon replacement or concurrently with other alterations, or via selection (Sherf et al., Zolotukhin et al., Cornelissen et al., and WO 99/14336), where codons are first replaced with preferred mammalian codons (Sherf et al., Zolotukhin et al., and WO 97/47358), rather than random codons (Pan et al.), plant codons (Hey et al.), or G and C residues (Cornelissen et al.), and in some instances, codons are further replaced to remove ATTTA sequences, splice sites, endonuclease cleavage sites, prokaryotic promoters, poly(A) signals,

prokaryotic factor-independent RNA polymerase terminators, inverted repeats, long runs of purines, RNA polymerase termination signals, TA and CG doublets and blocks of G or C residues of more than about 4 residues (WO 97/47358, Pan et al. and Hey et al.)

But at best, the cited documents, and given Applicant's disclosure as a "road map", suggest modifying a reporter gene over a large portion of the open reading frame with a view to remove undesired sequences introduced by codon replacement first with preferred mammalian codons, and then with other codons to remove ATTTA sequences, splice sites, endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, long runs of purines, RNA polymerase termination signals, TA and CG doublets and blocks of G or C residues of more than about 4 residues.

And while the cited documents may provide the motivation to repeat the alterations disclosed therein in a different gene, as there is no teaching or suggestion of Applicant's invention in the cited documents taken alone or in combination, the cited documents do not provide the motivation to arrive at Applicant's invention. That is because none of the cited documents recognizes that replacement of nonmammalian codons in a parent polynuclotide with mammalian codons introduces mammalian transcription factor binding sites not found in the parent sequence. Moreover, none of the cited documents suggests that a polynucleotide that is modified by replacement of nonmammalian codons with mammalian codons be further modified by replacement with other, lower usage mammalian codons to reduce the number of introduced mammalian transcription factor binding sites.

The Examiner is requested to consider that after codon optimization in conjunction with removal of non-transcription factor binding sites in click beetle and *Renilla* luciferase nucleotide sequences, Applicant identified about 100 and about 60 transcription factor binding sequences, respectively. Further codon replacement to remove those sequences yielded synthetic click beetle and *Renilla* luciferase sequences with about 50 and about 20 new transcription factor binding sites, respectively, i.e., they were introduced by codon replacement (Examples 1 and 3). The vast majority of the introduced sequences were subsequently removed to yield a synthetic nucleic acid molecule of the invention.

Moreover, one of skill in the art in possession of the cited documents would be required to identity transcription factor binding sites (Sherf et al. and possibly Cornelissen et al.), promoter sequences (Pan et al.), splice sites (WO 97/47358, Hey et al., and Pan et al.), and polyA sites (Hey et al. and Pan et al.), as sequences for removal by codon replacement, although the cited art would not lead the art worker to alter this combination of sites. Rather, Sherf et al. teach removal of internal palindromic sequences, restriction endonuclease sites, glycosylation sites, and transcription factor binding sites, WO 97/47358 discloses removing ATTTA sequences, inadvertent creation of intron splice sites, and unwanted restriction enzyme sites, Hey et al. disclose removing splice sites, polyA sequences, RNA polymerase termination signals, TA and CG doublets, and blocks of G or C residues, and Pan et al. disclose removing endonuclease cleavage sites, prokaryotic promoters, poly(A) signals, exon-intron boundaries, prokaryotic factor-independent RNA polymerase terminators, inverted repeats, and long runs of purines. Cornelissen et al. modify by changing A and T sequences to G and C sequences. Zolotukhin et al. and WO 99/14336 do not even mention removal of a set of specific regulatory sequences in a nonnative codon modified coding region.

Further, none of the cited documents discloses or suggests the use of software to identify particular regulatory sites, such as mammalian transcription factor binding sequences, in a database of transcription factor binding sequences.

In addition, one of ordinary skill in the art in possession of the cited art would have no reasonable expectation that any particular set of changes may improve activity in a gene that is to be expressed in a highly evolutionarily distinct cell. For instance, an increase in codon substitutions and a decrease in RNA destabilization sequences in a synthetic gene do not necessarily improve the transcriptional characteristics of the synthetic gene relative to the reference gene. In addition, it is unclear what changes to the HCV genes (WO 97/47358), *msp*-1 gene (Pan et al.) or *luc* (Sherf et al.) sequence result in improved activity in a heterologous host and why replacement of codons in *luc* with codons preferred in mammals and other alterations which resulted in *luc*$^+$ did not improve luciferase activity in all mammalian cells which expressed Luc$^+$.

The Examiner asserts that while it is true that none of the cited documents explicitly teach that codon replacements may create unwanted transcription factor binding sequences not

present in the wild-type sequence, Hey et al., WO 97/47358, and Pan et al. all show that the art recognized that codon modifications can introduce sequences which are unwanted within the synthetic gene and that additional codon modifications can decrease the introduction of those sequences, and that Sherf et al. clearly teach that the presence of transcription factor binding sequences within a reporter gene is an unwanted feature as it may interfere with the desired genetic neutrality of the reporter gene. The Examiner also asserts that it is obvious on its face that the more changes one makes, the higher the chances that such a detrimental sequence will be introduced, and that the remaining art clearly would have motivated one of skill in the art to make more substantial changes in codon preference within the luciferase of Sherf et al.

The Examiner's assertions are contradictory. Why would one of skill in the art make more changes that increase the chances that a detrimental sequence is introduced? Moreover, the template genes in WO 97/47358 and Pan et al. were already at least partially optimized for expression in humans, as HCV and *P. falciparum* replicate and express genes in humans. Further, Hey et al. teach changing the function of the protein encoded by the maize codon modified sequences.

In addition, it is likely relatively straightforward to remove a functional ATTTA sequence, splice site, selected restriction enzyme site, prokaryotic promoter sequence, poly(A) signal, RNA polymerase termination signals, prokaryotic factor-independent RNA polymerase terminator sequence or inverted repeat, or remove long runs of purines, TA and CG doublets, and blocks of G or C residues of more than about 4 residues (sequences disclosed as desirable to alter in WO 97/47358, Pan et al., and Hey et al.). In particular, perhaps only a single nucleotide replacement in a codon which forms part of a ATTTA sequence, intron splice site, restriction enzyme site, prokaryotic promoter, poly(A) signal, RNA polymerase termination signals, prokaryotic factor-independent RNA polymerase terminator, inverted repeat, long run of purines, TA and CG doublet, and block of G or C residues, without reference to adjacent sequences, may accomplish the removal of those undesired sequences.

In contrast, to remove a plurality of transcription factor binding sites, optionally in conjunction with other classes of sequences, by replacing codons, those modifications are selected in context, i.e., with reference to how those modifications impact adjacent sequences.

Accordingly, withdrawal of the § 103 rejections is appropriate and is respectfully requested.

CONCLUSION

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612) 373-6959 to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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CERTIFICATE UNDER 37 CFR § 1.8: The undersigned hereby certifies that this correspondence is being filed using the USPTO's electronic filing system EFS-Web, and is addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 12th day of February 2007.

Name: Dawn M. Poole Signature: _____